

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **09/328,975**

Confirmation No. **7574**

Applicants : **Jon A. Wolff, et al.**

Filed : **06/09/1999**

Art Unit : **1635**

Examiner : **Schnizer, Richard**

Docket No. : **Mirus009**

For: **Charge Reversal of Polyion Complexes**

Commissioner of Patents
PO Box 1450
Alexandria, VA 2231-1450

DECLARATION UNDER 37 C.F.R. ' 1.132

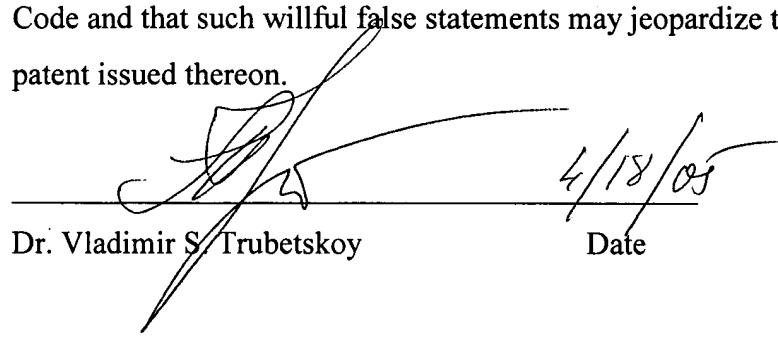
Dear Examiner:

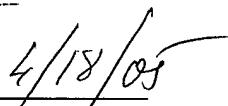
I, Vladimir Trubetskoy, hereby declare as follows:

1. I am an inventor of the captioned application.
2. The attached notebook pages (nos. 120-122) contain the experiment and data that is described in the specification in example 6 starting on page 26.
3. The abbreviation used throughout this notebook for polyaspartic acid was pAsp (see page 22 of the notebook). The abbreviation for polyacrylic acid used throughout the notebook was pAA (see page 111 of the notebook).
4. The polymer used in the experiment disclosed in the specification in example 6 was polyacrylic acid.
5. The abbreviation listed in the specification on page 22 line 27 for polyaspartic acid is a typographical error. The correct abbreviation for polyaspartic acid is found on page 6 line 26 and page 20 line 4 of the specification. Also note that the abbreviation pMAA, for

polymethylacrylic acid (see page 6 line 26 and page 20 line 4 and page 111 of the notebook), is consistent with PAA being the abbreviation for polyacrylic acid.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.


Dr. Vladimir S. Trubetskoy


Date
4/18/09

VIAL	43	237 LR	> Liver
#3	44	208 MC	
SpLL	45	311 Spleen	
	46	151 - lungs	
	47	128 - heart	
	48	184 - kidney	
	49	346 - tail	
SAMPLE		RLU	
VIAL	1	221 LR	> Liver
#3	2	234 MC	
SpLL	3	173 Spleen	
	4	156 Lung	
	5	141 heart	
	6	156 Kidney	
	7	395 tail	

VIAL	8	216 LR	
#4	9	142 MC	
	10	163 Spleen	
PEG XL	11	187 Lung	
	12	177 heart	
	13	413 kidney	
	14	221 tail	
VIAL	15	185 LR > LIVER	
#4	16	198 MC	
	17	161 Spleen	
PEG XL	18	162 heart	
	19	217 kidney	
	20	206 Kidney	
	21	217 tail	

In general, though all numbers are very low
non XL samples worked better (liver 5 times, tail)
So XL-particles are too stable

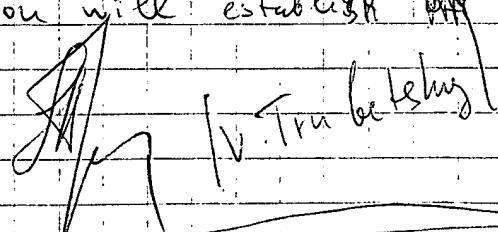
DNA/PLL34/PEG-SPLL particles did not aggregate upon
addition of salt. results in highest tail activity

2/10/99 Synthesis of PEG-pAsp conjugates.

• Polyaspartic acid (pAsp) $\xrightarrow{Mn^{2+} \text{ and } DQ}$ decondensing polyanion

• Idea) - To modify pAsp with increasing # of PEG chains to modify its ability to decondense DNA from its DNA/PLL complex

% of modification will establish the rate of DNA release



1 1 1

PLANT decondensation with different polyacrylates

Polyacrylates: PMAA 9 uM
 PAA 30 uM
 PPA ? uM Davis.

* DNA 10 μ g was condensed with 12 μ g PLL 34
 in 5 mM NEPPS pH 7.5.

No.	FI	[ug/ml]	10 μ g of each polyacrylate
1	-0.016	0.0118	were added to decondense
2	56.588	0.4026	DNA
3	8.452	0.0703	PLL 34
4	46.071	0.3300	PAA
5	56.180	0.3998	

No.	FI	[ug/ml]	10 μ g of PPA
1	-0.028	0.0118	DNA
2	53.121	0.3787	PLL 34
3	8.696	0.0720	PLL 34/PLL 34
4	54.085	0.3854	PAA

— this does not work

4.29.99 Cell-binding results of experiment on p. 110.

Polyanionic alone did not work - no cell binds.
 but probably concentration was too low

Particles:

(1) SP1210 vs SP1460 no XL - particles definitely disassemble on cell surface, SP1210 almost invisible only DNA (may be intensity), no internalization

460 particles are more stable than 210 (more colocalization).

(2) XL particles bind less than no-XL but with more colocalization (expected)

(3) wXL-460 one can see some SP1C binding.

1/6 in vivo: DNA/PEI/pAA

Samples recharged ~ μ g pAA before and after neutrality

2000/PEI/pAA

per animal: (1) 50 μ g / 100 μ g / 40 μ g(2) — 11 — 50 μ g + in 0.25 ml(3) — 11 — 60 μ g + (diluted) 4G(4) — 11 — 70 μ g

Results of in vitro binding exp. from So and Kirk

BNL
HeLa Kirk: all polyanions bind very weakly, more with RGD, less with PEG and SPCL

Particles bind more strongly with RGD more
PEG less, much more with SPCL

So: general binding less than usual (XL!)

with no difference from amount of SPCL.

5/6/99

Work with cyclic RGD peptide

RGD peptide (minus) GGC(RGD)MF-GC $M_w = 1000$

18 mg diss. in 1 ml MeOH + 5x of conc. HCl

add 100 μ l of T₂ solution in MeOH (50c)- in 5x increments \rightarrow until yellow color stays

Precipitate with 2+ 0

8 mg of peptide + 27.2 mg of Fmoc-PEG-NHS

diss. in 0.7 ml of 0.1M HEPES pH 8.0, 0.5M $NaCl$ disap. of Mg^2+ groups during reaction

S# 420.0

1 0.0003

2 0.0060

3 0.6759 before treat

10x 10.5 ml of Borax

after reaction - dial. ag. K_2O in 3.5 M $NaCl$
 MWCO overnight, $4^\circ C$.

folate particles for So:
 percent from p 114

(1) Rh DNA / PCC34 / Cg5SPCC210 - PEG folate 50/70/250

(2) — 11 — SPCC(SV)-PEG — 11 —

(3) — 11 — Cg5SPCC210 50/70/150

activated with 100/200 SDC/5-muS.

Results of 1/4 low pressure injections from p. 120

	#1	1548 LR		#2
VIAL		> liver		
1	1211 MC		20	6594 MC vial 2
2	1093 Spleen		21	2461 Spleen (210)
3	166931 lung		22	10074 lung Animal 5
4	2722 Heart		23	211 Heart
5	381 Kid.		24	136 Kidney
6	4041 LR	> liver	25	1374 iK
7	1986 MC		26	2538 MC
VIAL 8	6412 Spleen		27	2293 Spleen Animal 6
9	197443 lung		28	16544 lung vial 3 (210)
10	1649 Heart		29	196 Heart
50/100/40 11	916 kidney		30	178 kidney
12	one animal died 1/3		13	2951 LR one animal
			14	2290 MC died (1/4)
VIAL 15	1022 Spleen			
16	1557 Lung			
17	66 Heart			
18	12144 kidney			

31 11604 LC #3
 32 5822 MC 50/100/60
 33 225 Spleen Vial 3
 34 333 lung 7 days
 35 76 heart
 36 91 kidney
 37 21517 LK
 38 22765 MC Vial 3
 39 4404 Spleen Vial 3
 40 290 lung #5
 41 57 heart
 42 82 kidney
 43 12606 LR
 44 12913 MC Vial 3
 45 314 Spleen Vial 3
 46 352 lung #9
 47 95 heart
 48 67 kidney

Surv.

(10/3)

BKC

BKC

BKC

Vial 3

49 254 LR #4
 50 147 MC
 51 556 Spleen Vial 3
 52 70368 lung #4
 53 583 heart Animal #10
 54 139 kidney
 55 388 LK
 56 587 MC lung #10
 57 1507 Spleen heart #11
 58 150514 lung
 59 186 heart
 60 260 Spleen
 61 429 LK Animal #12
 62 407 MC
 63 1858 Spleen
 64 266123 lung
 65 3018 heart
 66 288 kidney

Surv.

(0/3)